3. TGase 2 assay. The expression and enzyme activity of TGase 2 was examined with western blot and immunocytochemistry. Total cellular proteins were isolated and western blotted as described previously. Enzyme activity was evaluated by determining the incorporated biotinylated pentylamine using horseradish peroxidase-conjugated streptavidin and measuring the absorbance at 492 nm using a microplate spectrophotometer.
Results: *1. RA up-regulated TGase 2 expression and enzyme activity in human chondrocytes.* Human chondrocytes treated with RA resulted in up-regulation of TGase 2 protein as shown by the western blot and immunocytochemistry. RA-induced TGase 2 expression increased time dependently. The enzyme activity of TGase 2 was also increased in RA treated cells compared with normal human chondrocyte cells.
2. TGase 2 induced by RA results in decreased apoptosis in human chondrocytes. Apoptosis in RA-treated chondrocytes was decreased, measured by Annexin-V FACS analysis, when compared with H_2O_2-treated cells chondrocytes. Similar patterns were observed in 3 independent experiments using chondrocytes obtained from different patients.
Conclusions: We have previously reported that endogenous TGase 2 expression was increased in human chondrocytes undergoing apoptosis. Inhibition of TGase 2 by MDC and TGase 2 siRNA was also shown to increase apoptosis and suggest a possible protective role of TGase 2 in chondrocyte apoptosis. The protective role of TGase 2 was further validated in this study as the RA-induced overexpression of TGase 2 decreased apoptosis of human chondrocytes. These results implicate a protective role of TGase 2 against apoptosis in human chondrocytes and the possibility of TGase 2 as a potential modulator of osteoarthritis.

192

VISFATIN/NAMPT: A POTENTIAL TARGET FOR NGF-TRIGGERED PAIN IN OSTEOARTHRITIS

S. Priam, E. Pecchi, M. Gosset, X. Houard, F. Berenbaum
Univ. Pierre et Marie Curie, Paris, France

Purpose: Obesity is the main risk factor for knee OA. The two main features of the pathophysiology of obesity-induced OA are based on a local component (mechanical stress) and a systemic component (pro-inflammatory adipokines). Nerve growth factor (NGF) is present within OA synovial fluid and may be involved in pain associated with OA. We previously showed that visfatin/NAMPT, an adipokine, has pro-degradative effects. However, its role in OA pain has not been evaluated yet.
Methods: Primary cultures of newborn mouse articular chondrocytes or cartilage explants were stimulated by increasing amounts of visfatin/NAMPT, IL-1 beta, prostaglandin E_2 (PGE_2) or by cyclic mechanical compression (0.5 Hz, 1 MPa). mRNA NGF levels were assessed by real-time quantitative PCR and NGF released into media was determined by ELISA.
Results: Unstimulated articular chondrocytes expressed low levels of NGF. Mechanical stress induced NGF mRNA expression and release in conditioned media. Visfatin/NAMPT, a pro-inflammatory adipokine produced by chondocytes in response to IL-1 beta, stimulated NGF expression (2 fold) and release (3.7 fold). When stimulated by IL-1 beta, a dose-dependent increase in NGF mRNA expression (5.7 fold increase with 10 ng/ml IL-1) and NGF release (x ng/ml, 19 fold increase with 10 ng/ml) in chondrocyte conditioned media was observed. Neither siRNA visfatin/NAMPT nor APO 866, an inhibitor of NAMPT enzymatic activity, prevented the production of NGF induced by IL-1 beta. Interestingly, PGE_2, which is produced by chondrocytes in response to IL-1 beta and visfatin/NAMPT, did not stimulate NGF production. Consistently, indomethacin, a cyclooxygenase inhibitor, did not counteract IL-1-induced NGF production.
Conclusions: These results suggest that obesity-induced OA pain may involve NGF mediated by the overexpression of visfatin/NAMPT and mechanical stress. These effects seem to be independent of the well-known pro-inflammatory mediators involved in OA pain, IL-1b and PGE2. Thus, along with reduction of weight, visfatin/NAMPT could be an interesting target for pain in OA

193

HUMAN OSTEOARTHRITIS CHONDROCYTES EXPRESS AND RESPOND TO THE PARATHYROID HORMONE RECEPTOR 1

T. Segovia-Silvestre, B.C. Sondergaard, H. Roashan, A. Goetrup, M.A. Karsdal, **A.-C. Bay-Jensen**
Nordic BioSci. A/S, Herlev, Denmark

Purpose: Parathyroid hormone (PTH) is an endogenous peptide hormone that elevates calcium levels by releasing calcium salts stored in bone and preventing their renal excretion. Although its role in bone metabolism has been thoroughly studied, little is known about its effects on cartilage. In this study, we sought to test the hypothesis that human chondrocytes do express the PTH 1 receptor (PTH1R) and are responsive to PTH *ex vivo*.
Methods: RNA was purified from freshly isolated, primary chondrocyte samples from OA knee cartilage. A dedicated reverse transcription polymerase chain reaction (RT-PCR) to amplify the coding region of the parathyroid hormone 1 receptor (PTH1R) mRNA was run. Similarly, western blotting was done with cell lysate; specific antibodies for the PTH1R protein were used to detect the presence of the receptor on human chondrocytes. In addition immune-cytochemistry as well as immune-histochemistry was done to identify the receptor in situ. Human chondrocytes were isolated and maintained serum-free for 1h in the presence of 100μM IBMX (PDE inhibitor), and subsequently stimulated with PTH 1nM-100nM + IBMX and the cAMP levels were quantified by ELISA. In addition, articular cartilage explants were cultured in 6 replicates for 17 days, with or without 10nM PTH treatment, and 5 μCi ^{35}sulphate was added for the last 24 hours. Soluble proteoglycans were released by 4M GuHCl and incorporated sulfate was measured. Neo-epitopes of pro-peptides of collagen type II (PIINP) were quantified as a measure of formation in the conditioned medium.
Results: We identified an approx. 2 kb band after RT-PCR, which was sequenced and aligned with the coding region of PTH1R mRNA. Furthermore, we detected a protein reacting against PTH1R antibody consistent with the transcript sequence in chondrocyte samples from osteoarthritis patients. PTH1R immunoreactivity was primarily localized in the cell membrane of chondrocytes, observed by immunochemistry. When stimulated with PTH, the cultured chondrocytes accumulated intracellular cAMP levels significantly ($P < 0.003$) in a dose-depend manner. The maximum concentration of PTH (100 nM) resulted in a 23-fold increase compared with baseline. In the explant cultures of OA articular cartilage, a two-fold increase of PIINP was observed in the supernatant after PTH stimulation when compared to non-stimulated cartilage samples. Furthermore, 10 nM PTH increased incorporation of ^{35}sulphate by 40% ($p=0.002$).
Conclusions: The current data strongly suggest that PTH, in addition to osteoblasts and bone turnover, also has direct anabolic effects on chondrocytes and cartilage. Human articular cartilage chondrocytes express both the mRNA and protein molecules of the parathyroid hormone 1 receptor. We have shown that PTH can not only avert but also facilitate cartilage generation in both in vitro and in vivo situations. Presented data indicate the potency of PTH and intrigues further investigation of PTH as a potential DMOAD.

194

REVERSIBILITY OF IMMOBILIZATION-INDUCED ARTICULAR CARTILAGE DEGENERATION AFTER REMOBILIZATION IN RAT KNEE JOINTS

A. Ando[1], H. Suda[1], Y. Hagiwara[2], Y. Onoda[1], E. Chimoto[1], Y. Saijo[3], E. Itoi[1]
[1]Tohoku Univ., Sendai, Japan; [2]Takeda Gen. Hosp., Aizuwakamatsu, Japan; [3]Tohoku Univ. of BioMed. Engineering, Sendai, Japan

Purpose: Joint immobilization (Im) causes articular cartilage degeneration. Im-induced cartilage degeneration is generally recognized as disuse atrophy caused by decreased chondrocytes activity. In our rat immobilized knee model with a plate and screws, the changes in the non-contact (NC) area was similar to disuse atrophy, but the changes in the transitional (T) area and contact (C) area were quite different. Reversibility of Im-induced cartilage degeneration is still controversial. The differences may be originated from methods of Im and measurement sites. The purpose of this study was to clarify the reversibility of Im-induced cartilage degeneration after remobilization.
Methods: Unilateral knee joints of adult male rats were rigidly immobilized at 150° of flexion with a plate and screws for 1, 2, and 4 weeks. After the experimental periods, the fixation devices were removed and the rats were allowed to move freely in standard cages for 16 weeks. Only screws were